

APPENDIX B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Patent Application of:)	Customer No.:	24239
Applicant:)	Docket No.:	014835-54.99-002
)		
Application No.:)	Examiner:	A. Boesen
)		
Filed:)	Art Unit:	1648
)		
Title:)	Confirmation	3193
)	No.:	
VIRUS COAT)		
PROTEIN/RECEPTOR)		
CHIMERAS AND METHODS)		
OF USE)		

**DECLARATION OF DR. ANTHONY L. DEVICO UNDER 35 U.S.C. §1.132 IN
U. S. PATENT APPLICATION NO. 09/684,026**

Mail Stop AF
Commissioner for Patents
P. O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Anthony L. DeVico hereby declare:

1. THAT I am a named co-inventor of the invention that is described and claimed in U.S. Patent Application No. 09/684,026 filed in the United States Patent and Trademark Office on October 6, 2000 in the names of Anthony L. DeVico, Timothy R. Fouts and Robert G. Tuskan for "VIRUS COAT PROTEIN/RECEPTOR CHIMERAS AND METHODS OF USE" (the "Application").
2. THAT, I am aware that the Application has been examined by the United States Patent and Trademark Office and that the March 15, 2007 Office Action issued by the United States Patent and Trademark Office included a rejection for lack of enablement.
3. That the present invention is described as chimeric polypeptides comprising a virus coat polypeptide sequence and viral receptor polypeptide sequence that are linked by an amino acid spacer, wherein the spacer is positioned between the virus coat polypeptide and the viral receptor polypeptide and linked thereto to form a single chain polypeptide. The amino acid sequence is of a sufficient length to allow folding of the single chain polypeptide to form an intramolecular interacting complex between the virus coat polypeptide and the viral receptor polypeptide.

4. That we have evaluated a chimeric polypeptide of the present invention to show the antibody response elicited by the chimeric polypeptide forms neutralizing antibodies. Since antibody responses against human CD4 can neutralize HIV, it is important to evaluate efficacy in a model where the endogenous CD4 is homologous to the CD4 sequences contained in the vaccine immunogen. In this case, the chances of inducing anti-CD4 response would be minimal as the animal would be tolerized to the CD4 moiety of the immunogen. This situation was addressed by immunizing rhesus macaques with a gp120-CD4 single chain that used rhesus CD4 in lieu of human CD4 thereby allowing evaluation of the concept in an autologous CD4 background. This allowed the mimicking of what would occur if FLSC was in humans and provided a more accurate indication of whether single chain complexes might break tolerance to CD4. The study also included a group of naïve animals and control groups that received either gp120_{BAL} or soluble human CD4 alone. This study also includes a rectal challenge with the heterologous R5 SHIV1_{62P3} to assess whether these responses would be in any way protective.
5. That after three immunizations (week 26), the majority of the complex-immunized macaques had circulating titers of neutralizing antibodies, as shown in Table 1 below.

Immunogen	Animal	Virus				
		BAL	92BR020	89.6	2044	SHIV _{162P3}
		Reciprocal ID ₉₀				
Gp120	829	<10	<10	<10	<10	<10
	830	14	<10	<10	<10	<10
	831	51	<10	<10	15	<10
	843	<10	<10	<10	16	<10
rhFLSC	833	<10	<10	<10	<10	<10
	835	42	141	32	>840	30
	837	45	103	28	>840	35
	841	75	>840	73	34	>840
sCD4	838	>840	>840	280	<10	>840
	844	20	23	14	63	23
	847	10	10	<10	85	<10
	851	67	>840	105	>840	>840
	Naïve	<10	<10	<10	<10	<10

Table 1 Neutralizing activity in Week 26 immune sera (collected after the third immunization). Titers were calculated versus control assays using matched preimmune sera. ID₉₀ = highest inhibitory dilution at which infection is reduced by 90 %. Mean values of quadruplicate assays are shown. ID₉₀ values > 1:50 are shaded grey.

At week 115, the animals were boosted, then, on week 119, challenged rectally with the heterologous R5 SHIV1_{62P3}. The resulting viral loads were tracked until week 135. Of note, the

neutralizing titers after the fourth immunization just prior to challenge did not reach the same levels as those observed after three immunizations (week 26) (data not shown). Despite this, the experiment produced three important observations. First, all animals vaccinated with rhFLSC exhibited accelerated clearance of plasma viremia (Figure 1 upper 4 graphs) and stronger suppression of tissue viremia (Figure 1 lower 4 graphs) (hereafter denoted as nonsterilizing "protection") compared to naïve controls and the other immunization groups (see Figure 1 and Table 2). Statistical comparison of the immunized groups is described in Table 2.

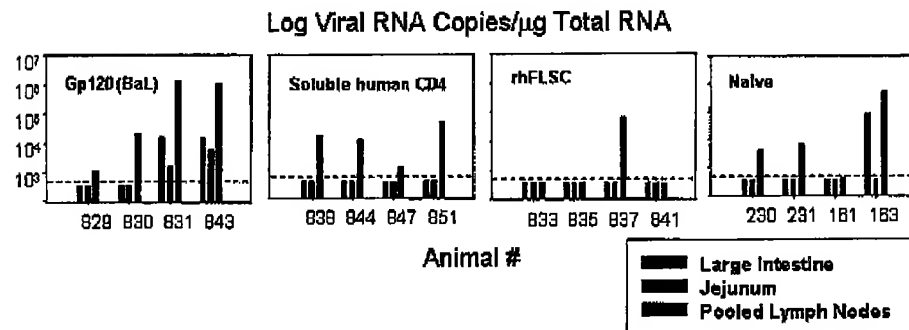
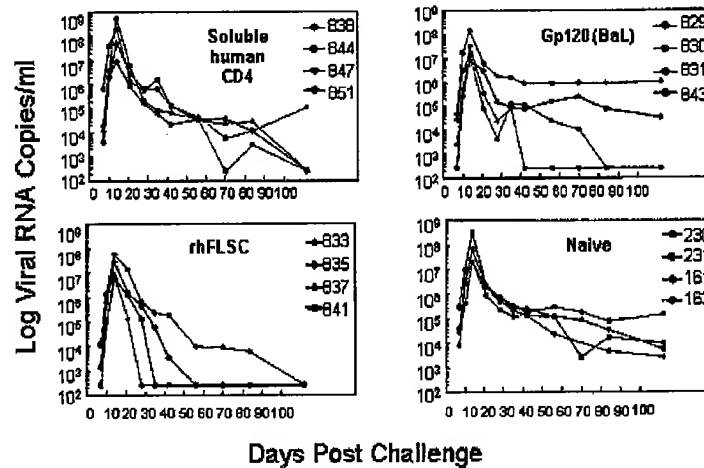


Table 2 Statistical comparison of viral load after challenge			
Statistical Test	rhFLSC group compared to		
	Naïve	shCD4	Gp120(BaL)
Area under curve (day 7 to 112)	p=0.006	p=0.013	p=0.103
Rate of decline from peak viremia (day 14 to 56)*	p=0.002 slope (β)= -0.35**	p=0.030 slope (β)= -0.25**	p=0.017 slope (β)= -0.32**
Mean area under the curve (day 14 to 112)	p=0.006	p=0.012	p=0.1
*Data were plotted between day 14 and 56 to maintain the assumption of linearity. The majority of rhFLSC vaccinated animals achieved undetectable viral load by day 56.			
**Difference in rate of decline between group C and the specified group. For example: β =-0.35 denotes 0.35log10 greater decline per day for the rhFLSC group compared to the naïve group.			

Single chain complexes elicit broadly neutralizing antibodies. High titers of these antibodies should afford heterologous protection against mucosal infection (see above). However, single chain gp120-CD4 complexes may elicit other response, which afford a form of nonsterilizing protection that is not clearly correlated with neutralizing antibody titers. These macaque studies show that the protective immunity afforded by certain DNA or DNA/MVA vaccines depends on "non-neutralizing" immune responses raised by the HIV envelope. Thus, the single chain complex has emerged as an envelope-based immunogen that has been used as a single subunit vaccine to afford protection against mucosal infection with a heterologous SHIV. These studies also raise the possibility that broadly neutralizing antibody responses (as detected by in vitro assays) might not be the only, or the most important, immunogenic feature of constrained gp120 structures.

6. THAT, in conclusion, the chimeras of the present invention have been found effective to raise neutralizing antibodies in an in vivo subject.

As a below-named declarant, I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements, and the like, so made are punishable by fine or imprisonment, or both, under Section 1001 or Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued therefrom.


 ANTHONY L. DEVICO, PHD

MAY 14, 2007
 DATE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Applicant: DeVico, et al.)	Customer No.: 24239
)	Docket No.: 014835-54.99-002
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Application No.: 09/684,026)	Examiner: A. Boesen
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Filed: October 6, 2000)	Art Unit: 1648
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Title: VIRUS COAT PROTEIN/RECEPTOR CHIMERAS AND METHODS OF USE)	Confirmation No.: 3193
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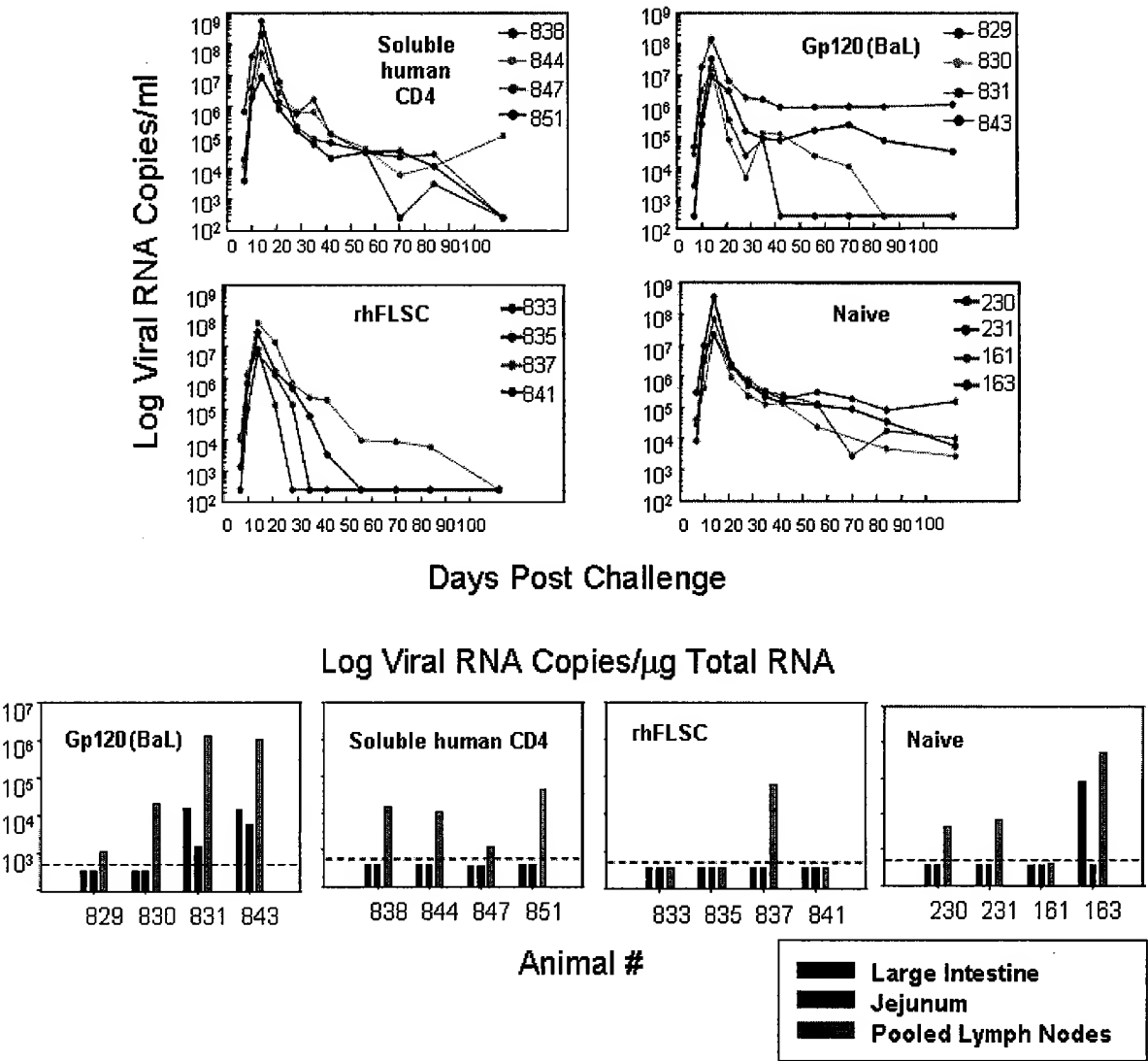


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ANTHONY L. DEVICO, PHD

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